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Rapid identification of the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera, Noctuidae) using species-specific primers in multiplex PCR

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The fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is a major pest native to the Americas. A recent invasion of FAWs from Africa eastward to South Asia, the Indochina Peninsula, and mainland China has received much attention due to the considerable economic losses in agriculture. FAWs can rapidly colonise a new area, likely due to the wide range of host plants, good flying capability, and high egg production. Therefore, a convenient, quick, and accurate tool for FAW identification is urgently required to establish a FAW invasion management strategy. In this study, FAW-specific primers were designed to recognise FAWs on the basis of internal transcribed spacer 1 (ITS1). The results revealed the accurate FAW recognition of the three congeneric species and eight common corn lepidopteran pests, especially at their larval stage. Furthermore, species-specific primers have confirmed their efficacy by using 69 FAW specimens from Taiwan, Thailand, and the United States, with a 96% success rate, excluding 3 decayed specimens. By using the simple, reliable, and convenient FAW-specific primers, a pest management programme can be developed not only to reduce sequencing costs and experimental time from 2 days to 4 h, but eradicate the FAW as soon as it enters a new area.

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), is a major pest native to the Americas with wide distribution from Canada to Argentina[1,2]. FAW is a highly polyphagous species that feeds on 76 families of host plants recorded in America, including more than 80 essential crops such as maize, sorghum, rice, cotton, alfalfa, and forage grasses[3,4]. Moreover, the FAW has a peculiar behaviour—it undergoes an annual long-distance migration, which has been proposed to be true for several lepidopteran taxa[1,2,5,6]. FAW adults migrate to southern Florida and Texas–Mexico in winter as they cannot survive the winter of Canada and the United States[1,7]; these two southern regions are also their native habitat. Thus, according to the biological features of FAWs, invasion events during a short interval are possible as well. Furthermore, the high volume of egg production by female FAWs—more than 1000 eggs during their lives—might be the factor for why FAW can colonise a new area and cause severe crop damage. Relevant reports concerning the severe economic losses caused by FAWs have been presented in various studies. For example, the damage caused by FAWs to economically essential crops such as maize, sorghum, rice, and sugarcane was estimated to be $13,383 million annually in Africa[8].

The migratory capability and wide range of host plants increase the survival possibility of highly reproductive FAW when it colonises in a new area, either through natural migration or anthropogenic activities. In 2016, FAWs became notorious after their invasion of Nigeria and Ghana[9,10] and then rapid intrusion, within 2 years, into > 20 sub-Saharan countries, causing severe economic losses of crops in Africa[11]. In 2018, FAW invaded

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